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TECHNICAL MANUSCRIPT 369

PROTECTIVE ACTION OF PROTEIN ON NEWLY GERMINATED BACILLUS ANTHRACIS SPORES IN TISSUE BREI

William I. Jones, Jr.
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MARCH 1967

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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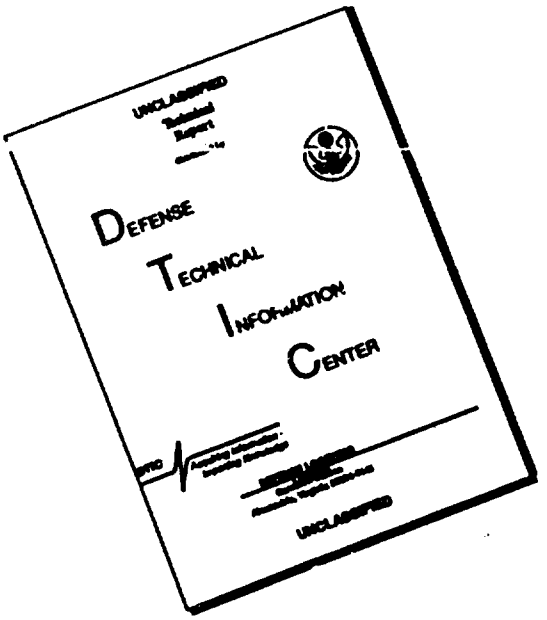

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Process Development Division
AGENT DEVELOPMENT AND ENGINEERING LABORATORY

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March 1967

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

PROTECTIVE ACTION OF PROTEIN ON NEWLY GERMINATED *BACILLUS ANTHRACIS* SPORES IN TISSUE BREI

ABSTRACT

A complete loss of viability was observed when newly germinated spores of *Bacillus anthracis* were placed in contact with freshly ground mouse tissue. This loss was overcome by the addition of 0.18% Bacto beef extract and 0.3% Bacto peptone to the amino acid germinating mixture.

While developing procedures for determining in vivo growth curves of *Bacillus anthracis*,[1] we observed that the conditions of germination of the spores markedly influenced the initial destruction of cells and our ability to account for the dose quantitatively.

Howie and Cruickshank[2] found viable anthrax spores in the spleen of mice killed 3 months after inoculation with *B. anthracis* spores. In order that dormant spores not be present in the host to affect the later quantitation of in vivo growth, a challenge with germinated spores was chosen. Heat-shocked spores were germinated in a medium containing 0.0125% L-alanine, 0.0125% L-tyrosine, and 0.00625% adenosine by placing 1×10^8 heat-shocked spores contained in one ml into a test tube containing 3 ml of the stock amino acid mixture and 6 ml of sterile distilled water.[3] The resulting suspension was incubated at 37 C for 30 minutes. Vegetative growth occurs if the spores are transferred to a suitable growth medium. The condition of the organism, i.e., dormant spore, germinated spore, or vegetative organism, was determined by phase microscopy and differential staining. The percentage germination was determined by differential plate count before and after the addition of sufficient phenol to obtain a final concentration of 1%.[4]

For this work, we believed it essential to be able to account, immediately after their introduction into the host, for 100% of the organisms used as inoculum. The steps following inoculation were to immediately sacrifice the animal, skin, grind in a Waring Blendor, then quantitatively dilute in gelatin phosphate buffer (pH 7.2) and plate onto nutrient agar. When spores were germinated in the amino acid mixture, no organisms could be recovered when assayed as described. To determine whether this observation was attributable to physical destruction of the organisms by the grinding mechanism or to some anthracidal material of normal tissue, germinated

spores were added to a suspension of uninfected (control) tissue and a viable plate count was made. Once again there was a complete loss of organisms. This finding was in complete agreement with that of Bloom et al.[5]

When 0.18% Bacto beef extract and 0.3% Bacto peptone were added to the amino acid mixture, the germinated spores were completely protected from the germicidal action of ground tissue or of tissue during grinding. Thus, we were able to account for and recover 100% of the organisms injected into a live animal.

This change of resistance in germinated spores may be due to a more advanced stage of development occurring in the presence of protein. As observed by light microscopy, there is no real difference in rate or percentage of spores losing refractivity in the two mixtures. Spores left in the amino acid mixture for 90 minutes will not develop into vegetative cells, whereas about 80 to 85% of the spores have divided in the protein-containing mixture. Because both dormant spores and newly germinated spores respond similarly, we suggest that resistance cells have developed the Embden-Meyerhof enzyme system, which is non-operative in dormant spores or in the initial stages of germinated spores.[6] However, other explanations are possible; for example, sensitivity to lysozyme may change in the presence of spermine.[7]

These data show that the newly germinated spore is very sensitive to destruction in the animal body. This fact needs to be considered in views on dynamics of infection, particularly in regard to host resistance and initiation of disease by different routes.

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